Studies on the effect of some concentrated solutions on the osmotic activity of plants*

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Turgidity, which is often of importance in bringing about rigidity in plant parts, is dependent to a great extent upon the osmotic pressure of the cell-sap, which keeps the cell-wall stretched to its greatest extent. The concentration of the cell-sap, upon which the osmotic pressure depends, varies according to the environment of the cell, and the permeability of the plasma-membranes.

Plasmolysis affords a means of studying the concentration of the cell-sap, and the changes which may be induced by the environment of the cell. If the cell lies in a solution more concentrated than the cell-sap, the greater osmotic pressure of the external solutes will cause a contraction of the protoplast. The protoplast will withdraw from the cell-wall to a greater or less extent, according to the increase or decrease of the difference between the external and internal osmotic pressures. It is this withdrawal of the protoplast from the cell-wall which constitutes plasmolysis.

In dealing with certain substances, glycerine, urea and acetamid, to which the plasma-membrane is more or less permeable, and which are considered harmless to the protoplast (Overton 17 and 18; Hampe 9; Beyer 2; de Vries 33; Klebs 10; Laurent 11; Meyer 14*b*), it was desired to ascertain if a stronger concentration of other substances would be necessary to bring about plasmolysis, after treatment with glycerine, urea, or acetamid, than under normal conditions.

METHODS AND EXPERIMENTS

In general, the experiments consisted in first determining the concentration of potassium nitrate, glycerine, urea, and acetamid isotonic with the cell-sap, namely such a solution as would just

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produce plasmolysis. When these isotonic concentrations were determined, specimens were placed in the required concentration of glycerine, urea, or acetamid, and left until turgidity was restored. They were then tested with potassium nitrate to determine the concentration necessary to replasmolyze them. The exact method of experiment differed according to the material used, and may best be described under each material.

In order that more accurate results might be obtained, normal solutions of the substances used were made. The solutions were made up on the basis of taking the number of grams represented by the molecular weight of the solute, dissolved in a little distilled water, and this solution diluted to one litre. A normal solution is designated by n, a one half normal by $n/2$, etc.

Philotria

The presence of numerous chloroplasts, as well as the more or less rectangular appearance of the cells in optical section, show incipient plasmolysis readily. The plants used had been in jars of water in the greenhouse for about two months. Fresh sprigs from these were kept in a glass of water, which was frequently changed, on a table near a south window. The importance of having good fresh material cannot be overestimated.

In experimenting with *Philotria*, whole leaves were broken from the stem with a small pair of forceps. This usually injured some of the basal cells, which were therefore not considered in determining the necessary concentration for plasmolysis.

The surplus water was removed from the leaf before applying the plasmolyzing solutions, in order to preserve the concentration of the solution used. Care was taken, however, not to remove too much water, lest the leaf should become flaccid, and vitiate the results. The leaf was mounted in a drop or two of the plasmolyzing solution, under a cover-glass. This solution was drawn off once or twice by filter-paper, and fresh solution added, both to keep the concentration correct and to bring about more rapid diffusion.

Potassium nitrate. Isotonic concentration. — As a result of several general tests, it was found that $n/4$ potassium nitrate would just produce plasmolysis in leaves of *Philotria*. This concentration

did not check cyclosis, and after two hours slow streaming was still visible.

Glycerine. (*a*) *Isotonic concentration.* — $n/3$ glycerine was the concentration required to produce plasmolysis in *Philotria*.

(*b*) *Increase in concentration of cell-sap.* — In order to determine the increase in the concentration (for potassium nitrate) due to the action of glycerine, a few leaves were placed in Van Tieghem cells filled with $n/3$ glycerine. After a lapse of some hours these were tested with solutions of potassium nitrate.

(1) $n/4$ potassium nitrate, the isotonic concentration, did not plasmolyze cells of *Philotria*, which had been in $n/3$ glycerine about five hours.

(2) It required $n/3$ potassium nitrate to plasmolyze cells which had been in $n/3$ glycerine five hours. The action was very uneven, showing complete plasmolysis in some cells and none in others, especially the apical cells. This was no doubt due to the greater permeability of the apical cells, which had therefore absorbed more glycerine.

(3) From $2n/5$ to $9n/20$ potassium nitrate was required to plasmolyze cells which had been in $n/3$ glycerine twenty-four hours.

The above experiments show that not only is the concentration of the cell-sap increased by the penetration of glycerine, but that the longer the action is allowed to go on the stronger the osmotic pressure of the cell-sap becomes.

Urea. (*a*) *Isotonic concentration.* — $n/4$ urea usually failed to plasmolyze cells of *Philotria*, but $n/3$ urea would plasmolyze nearly every cell.

(*b*) *Increase in concentration of cell-sap.* — Leaves were put in solutions in Van Tieghem cells, as in the above experiments with glycerine. Since $n/4$ urea sometimes produced plasmolysis, tests were made for increase in concentration of sap with both $n/4$ and $n/3$ urea.

(1) $n/3$ potassium nitrate produced only incipient plasmolysis in a few cells of leaves which had been in $n/4$ urea about twenty-four hours.

(2) $2n/5$ potassium nitrate was required to produce plasmolysis in such cells. The action was slow and gradual, varying from incipient to strong.

(3) $9n/20$ potassium nitrate was required to produce plasmolysis in leaves which had been in $n/3$ urea about seven hours.

Acetamid. — Since recovery from plasmolysis by acetamid usually occurs in a few minutes, the tests for increase in concentration of the sap were made upon the same cells soon after turgidity was restored.

(1) $n/3$ acetamid showed incipient plasmolysis. The cells were slowly replasmolyzed by $n/3$ potassium nitrate, incipiently at the apex, but stronger toward the base.

(3) $n/2$ acetamid plasmolyzed nearly all cells. It required from $n/3$ to $n/2$ potassium nitrate to replasmolyze such cells.

Tradescantia discolor

Free-hand sections were cut from the lower epidermis of *Tradescantia discolor*, whose cells have a red or violet-colored sap. These were mounted directly in the plasmolyzing solutions. The experiments showed a remarkable variation in the reaction of individual cells of a section, even in the same solution. In some merely incipient plasmolysis was produced, in others the action was quite strong. The cells over a vein were often more easily plasmolyzed than those between the veins.

Potassium nitrate. Isotonic concentration.—From $n/5$ to $n/6$ potassium nitrate was required to produce plasmolysis in *Tradescantia*. The action was more uniform with the $n/5$ solution.

Glycerine. (*a*) *Isotonic concentration.* — From $n/4$ to $n/5$ glycerine was necessary to produce plasmolysis. $n/5$ glycerine plasmolyzed most of the cells incipiently, whereas $n/4$ glycerine plasmolyzed all the cells clearly, some completely.

(*b*) *Increase in concentration of cell-sap.*

(1) In sections which had been in $n/5$ glycerine from six to eight hours, $n/5$ potassium nitrate showed no plasmolysis in most cells, though slight plasmolysis appeared in a few cells.

(2) $n/3$ potassium nitrate produced plasmolysis in such cells.

Urea. (*a*) *Isotonic concentration.* — $n/5$ to $n/6$ urea produced plasmolysis. The degree varied considerably with both solutions.

(*b*) *Increase in concentration of cell-sap.* — Sections of the lower epidermis of *Tradescantia* leaves were put in $n/5$ and in $n/6$ urea, and left seven or eight hours.

(1) $n/4$ potassium nitrate produced plasmolysis in the cells near a vein, but the action was very irregular.

(2) $n/3$ potassium nitrate produced plasmolysis in the cells as a whole.

(3) In sections which had been in $n/6$ urea about eight hours, $n/6$ potassium nitrate produced very slight plasmolysis in a few cells, but $n/5$ potassium nitrate plasmolyzed nearly all the cells, some quite strongly, thus showing that $n/6$ urea was not strong enough to increase the concentration of the sap.

Acetamid.

(1) $n/4$ acetamid was determined as the isotonic concentration. Sections which had been plasmolyzed by $n/4$ acetamid were replasmolyzed by $n/5$ potassium nitrate.

(2) $n/5$ acetamid plasmolyzed some cells, but the action was slow due to the weakness of the solution. $n/5$ potassium nitrate was required to plasmolyze such sections.

Beta vulgaris

Free-hand sections were cut from a red beet and mounted in a drop or two of the plasmolyzing solution, under a cover-glass. The cells near the epidermis were found best to work with, since they were of a deep-red color, and readily showed any change of position of the plasmatic membrane. But the paler parenchyma-cells were also considered.

Potassium nitrate. Isotonic concentration.

(1) $n/2$ potassium nitrate produced slight plasmolysis in some cells of red beet.

(2) $2n/3$ potassium nitrate produced plasmolysis in all cells; plasmolyzed strongly.

Glycerine. (*a*) *Isotonic concentration.* — $2n/3$ glycerine was found to be the isotonic concentration.

(*b*) *Increase in concentration of cell-sap.* — In carrying on experiments to determine the increase in concentration, sections of the beet were placed in small vials holding from 8 to 12 c.c. of solutions used. Turgidity was restored in five or six hours in sections of beet placed in $2n/3$ and also in $n/2$ glycerine.

(1) Cells which had been in $2n/3$ glycerine for six hours were replasmolyzed by $1.6n$ potassium nitrate. A few were replasmolyzed by $1.5n$ potassium nitrate.

(2) Those which had been in $n/2$ glycerine for six hours were replasmolyzed by $1.5n$ potassium nitrate; but the concentration increased later, so that $1.6n$ was required to produce plasmolysis.

The concentration did not exceed this in twenty-four hours.

Urea. (*a*) *Isotonic concentration.* — $3n/2$ urea just plasmolyzed the cells of red beet.

(*b*) *Increase in concentration of cell-sap.* — Sections which had been in $3n/2$ urea for seven hours, were replasmolyzed by $1.6n$ urea.

Acetamid. — $3n/2$ acetamid plasmolyzed these cells in about a minute. The action was a little stronger than with $3n/2$ urea.

Turgidity was usually restored in three to ten minutes.

(1) $3n/2$ potassium nitrate plasmolyzed these cells, slowly.

(2) $2n$ potassium nitrate produced too strong a plasmolysis.

(3) n potassium nitrate did not plasmolyze the cells at all.

Spirogyra

Fresh *Spirogyra* was obtained in April, and experiments carried on with this. A few threads were mounted on a slide, under cover-glass, in the plasmolyzing solution, which was drawn off two or three times by filter-paper, and fresh solutions applied. In testing for increase of concentration with glycerine, urea, and acetamid, some *Spirogyra* was put in the required solutions in small vials of about 15 c.c. capacity. In this way plenty of material could be used, and there was a better opportunity for obtaining uninjured threads.

Potassium nitrate. Isotonic concentration. — $n/5$ potassium nitrate usually produced slight plasmolysis, sometimes a stronger plasmolysis. In some cases, however, there was no plasmolysis produced by this concentration.

$n/4.5$ potassium nitrate produced plasmolysis in all cells. The degree varied from slight to rather strong, usually slight.

In these experiments, as in the others, there were usually one or two threads which were more difficult to plasmolyze than the others.

Glycerine. (*a*) *Isotonic concentration.*— $n/4$ glycerine produced incipient to slight plasmolysis in nearly all the cells. $n/3$ glycerine plasmolyzed all the cells.

In most of the cells the plasmolysis was slight, the protoplast withdrawing merely at the corners. In others it was stronger, so that the protoplast separated entirely from the end walls and formed ellipsoidal bodies.

(*b*) *Increase in concentration.*—It required $9n/20$ potassium nitrate to replasmolyze cells which had been in $n/4$ glycerine from four to five hours. $9n/20$ potassium nitrate plasmolyzed only some of the cells which had been in $n/3$ glycerine from four to five hours. $n/2$ potassium nitrate was required to replasmolyze all the cells. It required $2n/3$ potassium nitrate to replasmolyze cells which had been in $n/3$ or $n/4$ glycerine for twenty-four hours.

Urea. (*a*) *Isotonic concentration.* — $n/3$ urea plasmolyzed the cells incipiently or slightly.

$n/2$ urea produced a stronger plasmolysis in all the cells.

(*b*) *Increase in concentration.* — $n/2$ potassium nitrate was required to produced plasmolysis in cells which had been in $n/3$ urea from four to six hours.

$2n/3$ potassium nitrate was necessary to replasmolyze cells which had been in $n/2$ urea for five hours.

Most of the threads were too greatly injured to test the concentration after twenty-four hours.

Acetamid. (*a*) *Isotonic concentration.* — $2n/3$ acetamid seemed to be the isotonic concentration. The plasmolysis varied from incipient to medium strong, and recovery was usually rapid

$n/2$ acetamid produced only very slight plasmolysis in some of the cells.

(*b*) *Increase in concentration.*—It required $2n/3$ potassium nitrate to replasmolyze cells which had been in $2n/3$ acetamid from five to ten minutes.

$n/2$ potassium nitrate produced plasmolysis in cells which had been in $n/2$ acetamid from five to ten minutes.

Acetamid is very injurious in its action on *Spirogyra.* Many threads showed signs of injury in less than two minutes. Only those which seemed healthy and uninjured were considered in the above experiments.

SUMMARY

The results of the above experiments may be summarized in the following tables:

TABLE I

ISOTONIC CONCENTRATION

Plant.	Potassium nitrate.		Glycerine.		Urea.		Acetamid.	
	Normal.	%	Normal.	%	Normal.	%	Normal.	%
Philotria (leaf).	$\frac{1}{4}$	2.52	$\frac{1}{3}$	3.07	$\frac{1}{3}$	2.0	$\frac{1}{2}$–$\frac{1}{3}$	2.95–1.97
Tradescantia discolor (lower epidermis).	$\frac{1}{5}$–$\frac{1}{6}$	2.02–1.68	$\frac{1}{4}$–$\frac{1}{5}$	1.84–1.53	$\frac{1}{5}$–$\frac{1}{6}$	1.2–1.0	$\frac{1}{4}$–$\frac{1}{5}$	1.48–1.18
Beta vulgaris (root).	$\frac{2}{3}$	6.73	$\frac{2}{3}$	6.13	$\frac{3}{3}$	9.0	$\frac{3}{3}$	8.85
Spirogyra.	$\frac{1}{4.5}$	2.25	$\frac{1}{3}$	3.07	$\frac{1}{3}$–$\frac{1}{2}$	2.0–3.0	$\frac{2}{3}$	3.94

TABLE II

INCREASE IN CONCENTRATION OF CELL-SAP

Plant.	Plasmolyzing agent.	Concentration of plasmolyzing agent.		Duration of action.	Isotonic concentration of KNO_3.		Increase of KNO_3.	Increase in concentration of cell-sap.
		N.	%		N.	%	%	
Philotria.	Glycerine.	$\frac{1}{3}$	3.07	5 hrs.	$\frac{1}{3}$	3.36	0.84	1.33
		$\frac{1}{3}$	3.07	24 "	$\frac{9}{20}$	4.55	2.03	1.80
	Urea.	$\frac{1}{3}$	2.0	7 "	$\frac{9}{20}$	4.55	2.03	1.80
		$\frac{1}{4}$	1.5	24 "	$\frac{2}{5}$	4.04	1.52	1.60
	Acetamid.	$\frac{1}{2}$	2.95	15 min.	$\frac{1}{3}$–$\frac{1}{2}$	3.36–5.05	0.84–2.53	1.33–2.0
		$\frac{1}{3}$	1.97		$\frac{1}{3}$	3.36	0.84	1.32
Trades-cantia discolor.	Glycerine.	$\frac{1}{5}$	1.84	6 hrs.	$\frac{1}{5}$	3.36	1.34–1.68	1.66–2.0
	Urea.	$\frac{1}{5}$	1.2	7 "	$\frac{1}{5}$	2.02	0–0.34	1–1.2
		$\frac{1}{6}$	1.0	8 "	$\frac{1}{5}$	2.02	0–0.34	1–1.2
	Acetamid.	$\frac{1}{5}$	1.18	30 min.	$\frac{1}{5}$	2.02	0–0.34	1–1.2
		$\frac{1}{4}$	1.48	30 "	$\frac{1}{5}$	2.02	0–0.34	1–1.2
Beta vul-garis.	Glycerine.	$\frac{2}{3}$	6.13	6–24 hrs.	1.6	16.16	9.43	1.4
	Urea.	$\frac{3}{3}$	9.0	7 "	1.6	16.16	9.43	1.4
	Acetamid.	$\frac{3}{2}$	8.85	10–15 min.	$\frac{3}{2}$	15.15	9.42	1.6
Spirogyra.	Glycerine.	$\frac{1}{4}$	1.84	4–5 hrs.	$\frac{9}{20}$	4.55	2.30	2.02
		$\frac{1}{3}$	3.07	4–5 "	$\frac{1}{2}$	5.05	3.30	2.24
		$\frac{1}{4}$, $\frac{1}{3}$		24 "	$\frac{2}{3}$	6.73	4.48	2.99
	Urea.	$\frac{1}{3}$	2.0	4–6 "	$\frac{1}{2}$	5.05	2.80	2.24
		$\frac{1}{2}$	3.0	5 "	$\frac{2}{3}$	6.73	4.48	2.99
	Acetamid.	$\frac{2}{3}$	3.93	5–10 min.	$\frac{2}{3}$	6.73	4.48	2.99
		$\frac{1}{2}$	2.95		$\frac{1}{2}$	5.05	2.80	2.24

DISCUSSION

In carrying on plasmolytic experiments remarkable variability in the reaction of different cells, tissues, and plants may be observed. Yet there seem to be very definite influences at work which affect osmotic activity.

Variation in different plants. — It seems quite evident that plants may adapt themselves to environment in which it is difficult to obtain or to retain water, by increased concentration of the cell-sap. Pantanelli (19) quotes Cavaras to the effect that cold-enduring, salt and rock plants possess a very concentrated cell-sap. Ganong (8) remarks that there seems to be a close correspondence between halophilism of the plant and the power of its root-hairs to resist plasmolysis. The above experiments show that cells of the red beet have a much more concentrated cell-sap than *Tradescantia.*

Variation in different parts of plants. — De Vries (29) notes that the concentration necessary for plasmolysis differs for different parts or tissues of a plant, as well as for different plants. Apical cells have been generally considered to be much more difficult to plasmolyze than other cells, and the limit of concentration necessary to produce plasmolysis to decrease as the age of the cell increases (*cf.* Pfeffer 23, page 317; Ewart 6, page 12; de Vries 29). The above experiments with *Philotria* showed these variations clearly. The cells of the midrib were very difficult to plasmolyze, while those in adjoining parenchyma were usually among the first to show plasmolysis, as well as being plasmolyzed more strongly. But there seems to be some relation between the plasmolyzing substance in the substratum and the reaction of the apical and older cells, which would result from the varying permeability of the protoplast for different substances. In solutions of potassium nitrate and of urea, the cells near the midrib were plasmolyzed to a greater degree than the apical cells. The degree of plasmolysis, incipient in the apical cells gradually, increased in the older cells. With glycerine, however, the stronger plasmolysis occurred in the apical cells. But these cells, after turgidity had been restored, were the last to be replasmolyzed by potassium nitrate. Their greater permeability had allowed more of the glycerine to penetrate the protoplast, so that the concentration of the cell-sap was increased to a greater extent.

Permeability of plasma-membrane. — The permeability of the plasma membrane has been shown for many substances. Hampe (9), Beyer (2), de Vries (33), Klebs (10) and others have shown

that urea and glycerine readily penetrate the protoplast without injury. Klebs (10, page 540) states that glycerine not only penetrates the protoplast, but is there metamorphosed, and as shown by Meyer (14*b*) and Laurent may be converted into starch by certain higher plants; but, he continues, that the presence of glycerine in the cell-sap does not increase the turgor, since water-extraction by potassium nitrate took place normally.

Other experiments do not seem to bear out this view. De Vries found that the osmotic pressure was increased by urea (33*b*) and glycerine (33*a*) so that a much stronger concentration of other plasmolyzing solutions was required to induce plasmolysis again. Famintzin's experiments (7) with the culture of algae showed that by a gradual increase in the concentration of the medium a very considerable number of salts could be transferred into the cell-sap, and the cells in this way accustomed to stronger solutions.

The experiments in the present investigation showed, in nearly every case, an increase in the concentration of the cell-sap under the influence of glycerine, urea, and acetamid. Of the plants used in the experiments, *Tradescantia* showed the least concentration in cell-sap, and the least increase in concentration. Yet in some cases glycerine doubled the concentration of the sap, and the action in general was much stronger than with urea or acetamid. Urea increased the concentration in *Tradescantia* much less than in the other plants studied.

Relation between increase of concentration of sap, and concentration of solution. — There is considerable difference in the action of these substances. Although the final concentrations often do not show great differences, if these concentrations are compared with the original concentrations of the plasmolyzing substances, the greater increase in osmotic pressure due to glycerine will be brought out. The results with red beet cells may be cited as an example. The plasmolyzing concentration of glycerine was $2n/3$, that of urea $3n/2$ which gives a difference of $5n/6$. Yet the same or a less concentration of potassium nitrate was required to replasmolyze cells treated with these solutions, showing that glycerine was able to produce a comparatively greater osmotic pressure than urea.

This difference is much more marked in the case of acetamid.

The concentrations necessary to plasmolyze *Spirogyra* cells were $n/3$ glycerine, and $n/2$ acetamid. But the glycerine produced as great an osmotic pressure as the acetamid.

The action of acetamid in general seems to differ markedly from that of glycerine and urea. The latter substances produce a final concentration in the cell considerably higher than that of the plasmolyzing solution. For example, in the experiments just quoted the $n/3$ glycerine required an $n/2$ potassium nitrate to produce replasmolysis. But acetamid produces a final osmotic pressure equal to that of the solution used to plasmolyze the cells. An $n/2$ acetamid required an $n/2$ potassium nitrate to produce replasmolysis.

The above variations in the increase of concentration tend to show that the time necessary for the recovery of turgidity needs to be considered. After treatment with glycerine or urea, which require several hours for the plant to recover turgidity, a stronger concentration of potassium nitrate is required for replasmolysis, than after treatment with acetamid, in which the plant quickly regains turgidity. This was shown not only in *Philotria* but to a less extent in *Tradescantia*. — In *Philotria* it requires from $9n/20$ to $2n/5$ potassium nitrate to replasmolyze cells which have been plasmolyzed with $n/3$ solutions of glycerine or urea, whereas $n/3$ potassium nitrate produced plasmolysis in cells treated with $n/3$ acetamid. During the hours in which turgidity is being restored in cells plasmolyzed by glycerine or urea, osmotically active products may be formed in the cells. The short time required for turgidity to be restored in acetamid, as well as the fact that turgor is not raised above the osmotic pressure of the plasmolyzing solution, would seem to show that the increased turgor, in this case, was due merely to the penetration of the surrounding solution.

The strength of the solution used also has an influence on the increase of sap-concentration. A more concentrated solution will produce a greater increase in turgor than a weaker solution. The concentration of the sap in *Philotria* was increased 1.8 times in $n/3$ urea, but only 1.6 times in $n/4$ urea; or, expressed in terms of the increase in per cent. of potassium nitrate, 2.03 per cent. in $n/3$ urea, 1.52 per cent. in $n/4$ urea. Acetamid showed a marked difference in *Philotria*. In $n/2$ acetamid the concentration was

doubled, whereas in $n/3$ acetamid the concentration was only 1.33 times the normal sap. That, in general, there is an optimum concentration of the solution which will produce a maximum increase in turgor, and a maximum concentration of the solution beyond which no further increase in turgor will take place, and which may cause the death of the plant, has been shown by the experiments of Starge (26).

CONCLUSIONS

The concentration of cell-sap varies in different plants. It is comparatively weak in *Tradescantia discolor*, but much more concentrated in *Beta vulgaris*.

The limit of concentration necessary to produce plasmolysis varies in different parts and tissues of the plant according to the age of the cell, to the permeability of the protoplast, and to the plasmolyzing substance. With potassium nitrate and urea, the apical cells of *Philotria* were plasmolyzed incipiently, and the degree of plasmolysis gradually increased in the older cells. Glycerine, however, produced strongest plasmolysis in the apical cells. In all cases a stronger concentration of glycerine than of potassium nitrate was required to produce plasmolysis. Acetamid required the strongest concentration of the solutions used. The concentration of urea varied, but was sometimes stronger than that of glycerine.

The concentration of the cell-sap may be increased by the penetration of glycerine, urea, or acetamid. A comparison of the increase in concentration due to the various substances shows that glycerine produces relatively, and sometimes actually, the greatest increase. For, although, in some cases, as in *Philotria* and *Spirogyra*, urea and acetamid may produce a greater actual increase, this was due to the greater concentration of the plasmolyzing solutions. Acetamid cannot increase the concentration of the sap above the concentration of the plasmolyzing solution.

The duration of the action of the plasmolyzing solution influences the increase in the concentration of the sap. Glycerine and urea, whose action continues several hours, produce a relatively greater increase in the concentration than acetamid, whose action is very quick. The increase in the concentration of the sap of *Spirogyra* in solutions of glycerine was greater after twenty-four hours than after five hours.

The concentration of the plasmolyzing solution influences the increase in the concentration of sap. $n/2$ urea increased the concentration of sap in *Spirogyra* 2.99 times, whereas $n/3$ urea increased it 2.24 times. The greater increase takes place in the stronger concentration of the plasmolyzing solution.

This paper was submitted by Professor Atkinson to Professor Wilder D. Bancroft of the Chemical Department, who very kindly read it for the purpose of passing on the question of chemistry involved. He has suggested that the increase in concentration is due to the fact that the solutions, glycerine, urea, and acetamid pass through the plasma-membrane into the cell-sap, until the concentration of the solutes used is equal on both sides of the membrane, without reference to the concentration of other substances in the sap. Thus the total concentration in the sap may be equal to the original concentration of the sap plus the concentration of the glycerine, urea, or acetamid solutions used. Turgor would be restored when enough of the solution had penetrated so that the total concentration within the cell equaled the concentration in the external medium. If the plant remained in the solution after the turgor was restored, osmotic action might continue until the concentration of the solutes used would be equal on both sides of the membrane, and the total concentration would be greater than that of the external solution.

This hypothesis would explain the cases where the increase in concentration was equal to or less than the concentration of the plasmolyzing solution, as in the experiments with *Tradescantia* and *Philotria*, and all the experiments with acetamid. The increase in concentration above that of the external solution, amounting in *Beta* to 6.88 per cent., and in *Spirogyra* from 1.33–1.48 per cent. in glycerine solution, seems remarkable. In this connection reference may be made to the conclusion of Loeb (Studies in General Physiology, page 553) that "the simple osmotic theory of absorption which has been accepted by botanists cannot possibly be correct." This conclusion was based upon experiments with muscles, and *Fundulus*. "The fact that *Fundulus* can be thrown from sea-water into distilled water without any considerable swelling, or without any visible injurious effects, may find its explanation through the influence that various ions have upon the absorption of liquids."

Further investigations are needed to determine the causes for such an increase in concentration. Whether the substances undergo changes within the cell, and to what extent the changes take place, might be revealed in experiments upon growth of plants in the different solutions.

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